THE EFFECT OF OCCUPANCY ON THE GROWTH RATE OF TETRAHYMENA PYRIFORMIS IN A CLOSED BIOLOGICAL SYSTEM

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David Wilbur Lange

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David Wilbur Lange

Approved by Committee:

Rodney A. Rogers
Chairman

Paul C. Mueglitsch

Earl E. Gilkam

Dean of the Graduate Division

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CHAPTER I

INTRODUCTION

The growth pattern of a population is characterized by different phases; the lag phase, the log phase, the stationary phase, and the death phase. The relationship between the population and the changing environment is assumed in the growth pattern, as the population passes through the four phases. The number of individuals present insufficiently defines the course of the culture.

Presumably a closed biological system contains a definite amount of energy to be utilized. This suggests that some numerical value might be assigned to the closed system which could be used to describe the ability of a population to utilize the available energy. This does not imply that the organism's ability to utilize the available energy or the environment does not change, for it is sufficiently established that they do; but it suggests this to be a number which may be used in describing the changing ability of the population to utilize the shifting environment.

Meglitsch(1) suggests that the area beneath the growth curve is the total occupancy value of a given system, since this is essentially the summation of the total time that

all of the organisms lived in the given system.[1] The purpose of this investigation was to define population growth curves of Tetrahymena pyriformis, strain-W, and examine the areas beneath the growth curves in respect to occupancy.

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[1]P. A. Meglitsch, "Temperature and Growth Rates in Euplotes woodruffi Gaw," Proceedings of The Iowa Academy of Science, LXIII (December, 1956), 741-754. Reprint.

CHAPTER II

HISTORY

Abundant evidence dealing with the effects of crowding indicate that many factors may produce crowding in a population and that crowding decreases the rate of growth. Some evidence is available to indicate that crowding can have a stimulatory effect in the early stages of growth, but the final result is a decreased growth rate.

Myers(2) working with paramecia concluded that populations from small inoculations rise rapidly to a peak.[1] In one milliliter of a culture medium, inoculations of one, two, and four organisms advanced essentially to the same population peak, while inoculations of eight organisms produced population peaks of one-half that of the smaller seedings. The time required to reach the population peak is greater as the inoculations become smaller.

Chapman(2) demonstrated that the exhaustion of the food supply is not necessarily the limiting factor of the maximum population.[2] By introducing varying numbers of

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[1] W. C. Allee, Animal Aggregations (Chicago: University of Chicago Press, 1931), p. 136.

[2] Ibid., p. 138.

beetles to a definite amount of flour, and renewing the food supply each time a population count was made, he found that a definite number of organisms develop in a unit volume of culture medium.

Gause(3) recognized that the growth of organisms is sometimes subject to definite quantitative laws and implied that the process is extremely complicated, and not always in agreement with the predictions of relatively simple mathematical theory.[1]

Following the principals of Andre' Lwoff, Elliott(4) succeeded in isolating the ciliate Colpidium striatum (Tetrahymena geleii) in an axenic medium.[2] Taxonomic confusion at this time ended when W. H. Furgason(4) identified Elliot's ciliate as Tetrahymena geleii.[3] J. O. Corliss(4) later clarified the relationships between the Colpidium-Glaucoma-Leucophrys-Tetrahymena groups.[4] G. W. Kidder(4) and his associates worked out the amino acid and vitamin requirements of Tetrahymena pyriformis,strain-W, and Elliott studied the requirements for Tetrahymena pyriformis, strain E.[5]

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[1]G. F. Gause, The Struggle for Existence (Baltimore: The Williams and Wilkins Company, 1934), p. vii.

[2]Alfred M. Elliott, "A Quarter Century Exploring Tetrahymens," The Journal of Protozoology, VI (February, 1959), 1. Reprint.

[3]Ibid., p. 2.

[4]Ibid.

[5]Ibid.

These nutritional landmarks opened the door for a multitude of metabolic studies. Elliott and Hogg(4) concluded from their studies that the basic metabolic pathways of Tetrahymena pyriformis and higher animals are similar.[1]

Phelps(5) points out that the work of early experimenters did not employ organisms in pure culture. The results of non-pure cultures were contradictory.[2] Phelps reinvestigated protozoan growth in pure culture and established growth rates at temperature ranges similar to previous workers. Phelps and Kidder(6) both agree that the growth rate of Tetrahymena pyriformis in pure culture during the logarithmic growth phase is perfectly regular and consistent.[3] Kidder then concluded that a conditioned medium, medium which has supported a population of ciliates, possesses the power to accelerate or inhibit the growth of a succeeding population. Removal of the previous population by centrifugation causes acceleration of growth, while removal of the previous population by filtering causes inhibition of growth. A conditioned

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[1] Ibid.

[2] Austin Phelps, "Growth of Protozoa in Pure Culture," The Journal of Experimental Zoology, CII (1946), 277-292.

[3] George W. Kidder, "Growth Studies on Ciliates. V. The Acceleration and Inhibition of Ciliate Growth in Biologically Conditioned Medium," The Journal of Physiological Zoology, XIV, No. 2, (April, 1941) 209-226.

medium treated with sterile charcoal or plaster of paris produces the same effect as the filtered medium. The accelerating factor decreases after the population age passes sixty hours; also the conditioning effects are destroyed by heat and are lost through standing.[1]

In formulating the "Division Index"(the visible stage of cytoplasmic fission expressed on a percentual basis of the population number), Scherbaum(7) agrees with Phelps and Kidder by concluding that the higher index obtained in the early logarithmic phase of growth is due to the prolongation of cell fission rather than a synchronization of the cells.[2]

Robertson(10) observed that certain ciliates (Enchelys and Colpoda), when introduced into small volumes of fresh culture medium, demonstrate an increased fission rate. By hypothesizing a nuclear autocatalyst, he attempted to explain the increased fission rate to be dependent upon the amount of the autocatalyst present in the nucleus. He reasoned that as the size of a population increased, the amount of the autocatalyst in the nucleus of the individual increased, since each individual would contribute less of the autocatalyst to

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[1] Ibid.

[2] Otto Scherbaum, "The Division Index and Multiplication in a Mass Culture of Tetrahymena Following Inoculation," The Journal of Protozoology, XII (November, 1957), 257-259.

the culture medium due to the low solubility of the autocatalyst in the culture medium.[1] Other workers were able to corroborate Robertson's observations but failed to give evidence for his explanation.[2]

A steady shift in the physiological state of cells and of the medium constitution was demonstrated by Prescott(7) during the logarithmic and stationary phases of culture growth. He concluded that the total logarithmic period is not physiologically homogeneous even though the average cell generation time remains constant.[3]

Meglitsch in an attempt to define more accurately the capacity of a culture to utilize the environmental potential of a given environment, used the area beneath the growth curve as a value of total occupancy.[4] Promoting the idea that the causes of events are prior to the events themselves, he postulated that the growth rate of an organism is more dependent upon prior occupancy than any other population growth factor.[5]

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[1]W. C. Allee and others, Principles of Animal Ecology (Philadelphia and London: W. B. Saunders Company, 1949), pp. 357-360.

[2]Ibid.

[3]D. M. Prescott, "Change in the Physiological State of a Cell Population as a Function of Culture Growth and Age," Experimental Cell Research, XII (February, 1957), 126-134.

[4]Op. cit., p. 750. [5]Op. cit., p. 753.

CHAPTER III

PROCEDURE

The experimental objective demanded that the population growth curves of Tetrahymena pyriformis be exactly defined. Complete cell counts during the entire period of occupancy would be the most acceptable. Instead of this, estimates of the number of cells present in cultures were made by the determination of the optical density of the cultures. The percent of light transmitted through the culture medium was used as an index to determine the numbers of organisms present in the cultures. By recording the percent of light transmission with a Bausch and Lomb "Spectronic 20" colorimeter at various times, the growth curves could be determined.

Specimens of Tetrahymena pyriformis, strain-W, obtained from the Department of Zoology, State University of Iowa, Iowa City, Iowa were cultured in bacteria-free Loefer's Medium.[1] The medium was prepared by dissolving in one liter of double distilled water, fifteen grams of Bacto-proteose peptone(Difco Laboratories), five grams of Bacto-casitone(Difco Laboratories), five grams of dextrose, two grams of sodium

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[1]Loefer's Medium formula was obtained from the State University of Iowa, Iowa City, Iowa. This is the same medium the Department of Zoology uses to maintain stock cultures of Tetrahymena pyriformis.

chloride(NaCl), one gram of dibasic potassium phosphate (K_2HPO_4), one gram of dibasic sodium phosphate(Na_2HPO_4), five-tenths of one gram of Bacto-yeast extract(Difco Laboratories), and three-tenths of one gram of magnesium chloride($MgCl_2$).

The medium was then autoclaved at a minimum of fifteen pounds pressure for fifteen minutes. The phosphates present buffered the medium at pH 7.4. The pH of the medium was recorded using a Coleman pH meter. The protozoans will grow in proteose peptone plus the salts, but the addition of yeast extract improves their growth.

Stock cultures of Tetrahymena pyriformis were grown in one-hundred milliliters of Loefer's medium at room temperature, in a two-hundred-fifty milliliter florence flask, which served as a source of organisms used in inoculating the growth flasks. Twenty to thirty hours before the inoculation of the growth flasks, one bacteriological loop-full of the stock culture was transferred into fifty milliliters of the experimental medium in a two-hundred-fifty milliliter florence flask. This culture was then incubated at thirty seven degrees Celsius until the inoculation of the growth flasks.

The erlenmeyer growth flasks used in this investigation were modified by having a pyrex test tube with a one-half inch diameter, opening into the inside of the flasks,

attached approximately four centimeters from the top of the flask. The erlenmeyer growth flasks were stoppered with cotton. By tipping the modified flasks, the culture medium in the flasks containing the protozoans would fill the test tube and the percent transmission readings could be read directly without the removal of a sample from the growth flask.

Inoculation of the growth flasks consisted of aseptically transferring one bacteriological loop-full of the subculture into each of the growth flasks. The temperature of the growth flasks was held constant at thirty-seven degrees Celsius in an "Imperial 400" incubator produced by the Chicago Surgical and Electrical Company, except during the counting of the cultures. The large surface area of the two-hundred-fifty milliliter flasks provided a rapid gas exchange with the atmosphere.

Accurate determination of the percent transmission required that the growth flasks be matched. A cobalt chloride stock solution was prepared by dissolving twenty-two and five-tenths grams of cobalt chloride in one liter of a one percent hydrochloric acid solution. The test tubes of the modified flasks were then filled with the cobalt chloride stock solution. Setting the wavelength scale of the colorimeter to five-hundred-forty millimicrons, one flask was

chosen as a reference flask, and the colorimeter was adjusted to give a fifty percent transmission reading with this flask. Using this flask to periodically check the fifty percent transmission reading, the transmission readings of the other flasks were then recorded. Flasks with less than a one percent transmission deviation from the fifty percent transmission of the reference flask were retained for the experimental growth flasks. The ratio of the percent transmission of the reference flask to the percent transmission of the unknown flask provided a constant (k) which corrected the reading of the unknown flasks to the reading of the reference flask. Photometric curves of Loefer's Medium were drawn by plotting the percent transmission of the medium against different wavelengths of light. In colorimetry, minimum transmittance is preferred, since the greatest sensitivity is obtained at the absorption maximum. In this case maximum transmittance was used to reduce any error which might be introduced by a change in the transmittance of the medium. Plots of the percent transmission of centrifuged medium before and after growth, against different wavelengths of light, were made on a portion of the absorption curve which had very little slope(eight-hundred millimicrons) and no change was indicated in the transmission of the medium.

Dense cultures of Tetrahymena, after shaking to insure

equal distribution, were divided into two aliquots. One aliquot was centrifuged for six minutes in order to obtain a diluting medium. Using a seriological pipette, five milliliters of the second aliquot were transferred into the attached test tube of one of the growth flasks and the percent transmission was recorded. One milliliter of the diluting medium was added to a second growth flask, with four milliliters of the five milliliters in the first growth flask, and the percent transmission was recorded. This serial dilution of four milliliters of the previous dilution to one milliliter of the diluting medium was continued until eighty percent transmission readings were obtained. Then part of the second aliquot was transferred to a test tube, and the test tube was placed in hot water for one minute. This killed the protozoans present. After cooling, a blood pipette was used to transfer part of the dead organisms to a Levy-Hausser Corpuscle Counting Chamber(Clay-Adams, Inc.). This counting chamber consists of nine, one square millimeter areas. Five separate counts of the dead organisms were made over the entire nine square millimeters of area. The five counts were recorded and averaged. Estimates of the number of organisms per milliliter of culture medium were derived using the average count of the nine square millimeter areas. The number of organisms in each serial

dilution was determined from the estimate of the number of organisms per milliliter of the culture medium, and was associated with the appropriate serial dilution. Nine cultures were counted using this method.

Plots of the number of organisms against the percent transmission of the serial dilutions on semi-log paper suggested that a logarithmic mathematical relationship existed. Using the method of lease squares, the relationship

$$\log y = 3.3442 - 0.183x$$

was determined, where y is the number of organisms, and x is the percent transmission. The corrected standard deviation of y on x is 10.8. The probable error of y on x is 7.2. The coefficient of correlation, Rho, is 0.997. Since the correlation coefficient approaches one, and the size of the sample is small; in order to test if the observed correlation coefficient differs significantly from a given theoretical value expected of the sample size, the value of a Z-transformation was determined. The Z-transformation value of 3 ± 0.63975 is in complete agreement with Rho, placing Rho between 0.98233 and 0.99817. The Rho value observed is not significant.[1]

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[1] R. A. Fisher, Statistical Methods for Research Workers (London: Oliver and Boyd, 1934), pp. 183-192.

Two variables were used in the investigation of the growth process. First the concentration of the medium was held constant while the volume of the culture was varied. Second the volume of the cultures was held constant while the concentration of the medium was varied.

The data for growth in different volumes of medium was recorded at eight hundred millimicron wavelengths of light, by growing Tetrahymena pyriformis in seventy-five, fifty, and twenty-five milliliters of medium. The percent transmission of light of the cultures was recorded each morning and evening with approximately a twenty-four hour interval between the morning readings of each day for a two-hundred-sixty hour period. By growing the organisms in fifty milliliters of medium, and varying the concentration of the medium by full-strength, one-half strength, and one-quarter strength, data for the growth of the organisms in different concentrations of the medium was obtained in the same manner as was done with the different volumes.

At the termination of each period of growth, Bacto-nutrient agar(Difco Laboratories) slants were prepared and inoculated with one bacteriological loop-full of the culture to establish that the cultures were not contaminated.

CHAPTER IV

DATA AND EXPERIMENTAL RESULTS

The percent of light transmission of the medium was converted to the number of organisms per milliliter from the calibration curve. The data were grouped into ten hour intervals and the arithmetic mean was determined for each ten hour period. Specific growth rates for each ten hour period were then determined. Areas beneath the population curves were derived from the number of organisms per milliliter using Simpson's Rule. The log to the base two was determined for the mean figures and graphs were constructed. The results for the growth of Tetrahymena pyriformis in different volumes of medium of the same concentration are summarized in Table I and Figure I.

Examination of the results as summarized in Table I indicate that the seventy-five milliliter cultures reach a maximum of two-hundred-thousand organisms per milliliter, the fifty milliliter cultures reach a maximum near four hundred thousand organisms per milliliter, and the twenty-five milliliter cultures reach a maximum near eight hundred thousand organisms per milliliter. Specific growth rates for the seventy-five and fifty milliliter cultures advance to the same peak, but the specific growth rate of the twenty-five

TABLE I

GROWTH OF TETRAHYMENA PYRIFORMIS IN DIFFERENT VOLUMES OF MEDIUM OF THE SAME CONCENTRATION IN THOUSANDS PER MILLILITER

Time Periods 10 hrs. each	Average $\bar{N}$ for 75 ml	$\frac{\Delta N}{t}$	$\frac{\Delta N/\Delta t}{N}$	Area	Average $\bar{N}$ for 50 ml	$\frac{\Delta N}{t}$	$\frac{\Delta N/\Delta t}{N}$	Area	Average $\bar{N}$ for 25 ml	$\frac{\Delta N}{t}$	$\frac{\Delta N/\Delta t}{N}$	Area
1	39.8	0.0	0.00		37.9	1.8	0.05		40.2	- 1.2	-0.03	
2	39.8	2.7	0.68		39.7	0.7	0.00		39.0	4	0.10	
3	42.5	47.7	1.12	80	40.4	45.0	1.11	79	43.0	79	1.84	79
4	90.2	10.8	0.11	140	85.4	55.6	0.65	136	122	98	0.80	151
5	101	33.0	0.33	248	141	53.0	0.37	253	220	154	0.70	330
6	134	26.0	0.19	350	194	63.0	0.32	391	374	43	0.11	565
7	160	15.0	0.09	508	257	41.0	0.16	644	417	128	0.30	1041
8	175	17.0	0.10	650	298	20.0	0.07	871	545	56	0.10	1429
9	192	20.0	0.10	858	318	22.0	0.07	1233	601	67	0.11	2107
10	212	- 6.0	-0.03	1031	340	12.0	0.03	1508	668	44	0.06	2627
11	206	- 3.0	-0.01	1274	352	3.0	0.01	1910	712	41	0.06	3435
12	203	0.0	0.00	1454	355	6.0	0.01	2213	753	20	0.02	4046
13	203	- 9.0	-0.04	1681	361	18.0	0.05	2621	773	-19	-0.02	4934
14	194	- 6.0	-0.03	1861	379	19.0	0.05	2943	754	-66	-0.09	5586
15	188	- 60.0	-0.32	2070	398	13.0	0.03	3379	688	-90	-0.13	6426
16	228	- 28.0	-0.12	2259	411	4.0	0.01	3732	598	-81	-0.13	7011
17	200	- 4.0	-0.02	2503	415	7.0	0.02	4198	517	-108	-0.21	7625
18	196	6.0	0.03	2688	422	-24.0	-0.06	4567	409	-72	-0.17	8 093
19	202	- 9.0	-0.04	2898	398	-11.0	-0.03	5032	337	-17	-0.05	8455
20	211	16.0	0.07	3090	387	-34.0	-0.09	5388	320	-37	-0.11	8829
21	195	- 14.0	-0.07	3282	353	-16.0	-0.04	5798	283	-28	-0.10	9089
22	209	21.0	0.10	3472	337	-27.0	-0.08	6125	255	0	0.00	9417
23	230	- 15.0	-0.06	3702	310	-26.0	-0.08	6469	255	102	0.40	9608
24	215	- 25.0	-0.12	3902	284	-47.0	-0.16	6765	357	11	0.03	9956
25	190	37.0	0.19	4129	237	43.0	0.18	7030	368	11	0.03	10,292
26	227			4326	280			7299	379			10,683

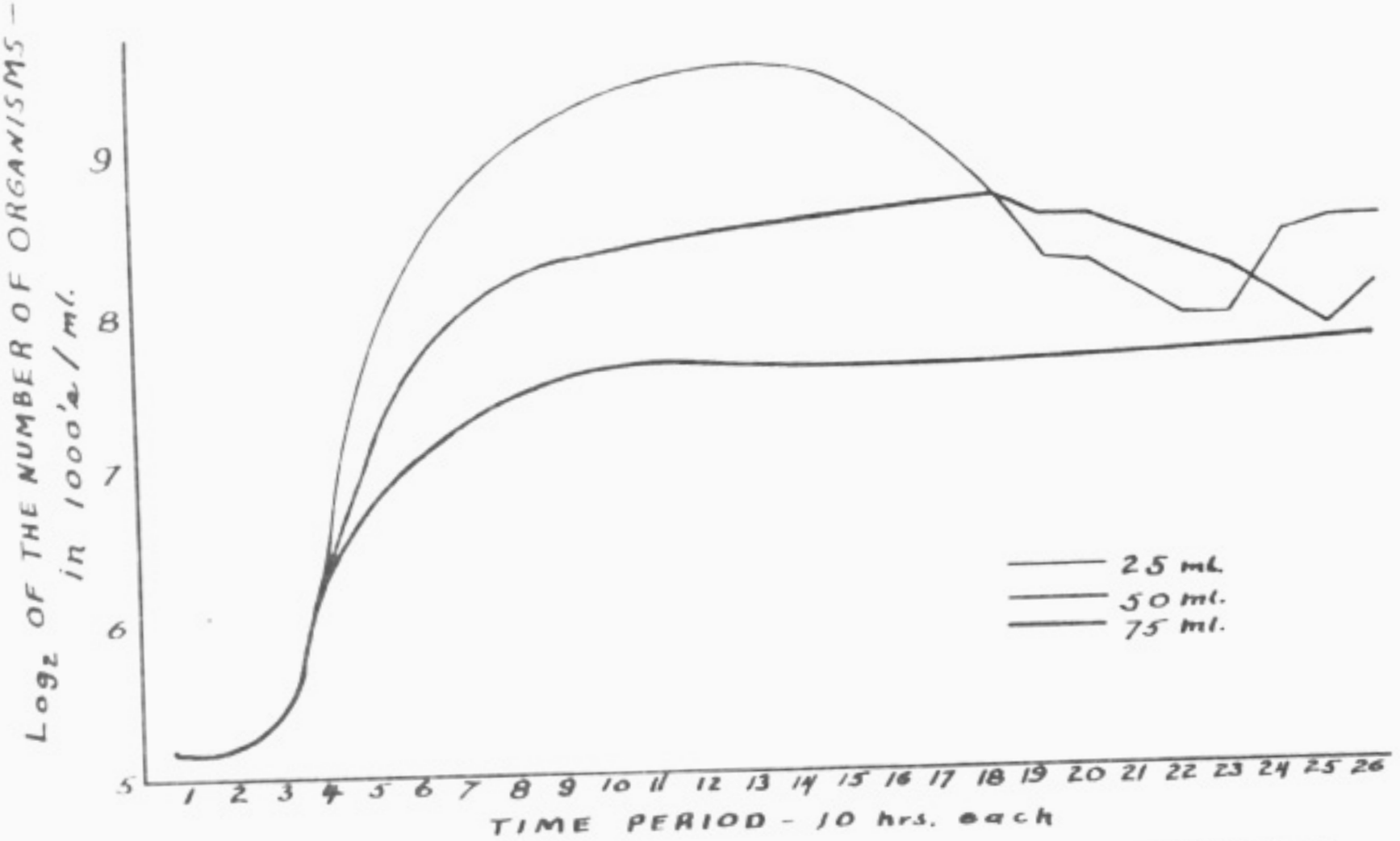

Figure 1. Growth of *Tetrahymena pyriformis* in different Volumes of medium of the same concentration.

milliliter culture attains a higher peak. A general trend toward a higher specific growth rate is observed in the twenty five milliliter culture, while the seventy-five milliliter culture tends to manifest the lower specific growth rate.

At the same time period after inoculation, the occupancy values of the twenty-five milliliter cultures are greater than the occupany values of the fifty milliliter cultures, and the occupancy values of the fifty milliliter cultures are greater than the occupancy values of the seventy-five milliliter cultures.

The growth observed in equal volumes of different concentrations of the medium are summarized in Table II and Figure II. An examination of the results as summarized in Table II reveals that the full-strength and the half-strength medium attain similar plateaus. The quarter-strength medium arrives at a plateau approximately one generation below the plateaus of the full and half-strength mediums. At the population peak oscillation of the population density is characteristic of the three cultures. A greater oscillation of the population density than indicated by the probable error is observed in the quarter-strength medium at fifty hours of growth. Between fifty and one-hundred-twenty hours of growth the greater oscillations of population density of the quarter strength medium remain near the population peak.

TABLE II

GROWTH OF TETRAHYMENA PYRIFORMIS IN DIFFERENT CONCENTRATIONS OF MEDIUM OF THE SAME VOLUME IN THOUSANDS PER MILLILITER

Time Periods 10 hrs. each	Average $\bar{N}$ for Full Strength	$\frac{\Delta N}{t}$	$\frac{\Delta N/\Delta t}{N}$	Area	Average $\bar{N}$ for Half Strength	$\frac{\Delta N}{t}$	$\frac{\Delta N/\Delta t}{N}$	Area	Average $\bar{N}$ for Quarter Strength	$\frac{\Delta N}{t}$	$\frac{\Delta N/\Delta t}{N}$	Area
1	39.4	0.1	0.00		38.1	15	0.39		38.7	10.1	0.26	
2	39.5	9.8	0.25		53.1	39.8	0.75		48.8	29.1	0.60	
3	49.3	44.7	0.91	82	92.9	83.1	0.89	114	77.9	51.1	0.65	103
4	104	36.0	0.35	150	176	82	0.46	229	129	42.0	0.32	194
5	140	57.0	0.41	284	258	57	0.22	466	171	-32	-0.18	358
6	197	26	0.13	424	315	25	0.08	704	139	35	0.25	495
7	223	45	0.20	667	340	17	0.05	1085	174	-19	-0.11	659
8	268	29	0.11	872	357	12	0.03	1383	155	23	0.15	807
9	297	11	0.04	1198	369	11	0.00	1697	178	12	0.07	983
10	308	17	0.05	1453	370	3	0.00	2018	190	-23	-0.12	1148
11	325	12	0.04	1816	373	- 5	-0.01	2438	167	15	0.09	1351
12	337	11	0.03	2100	368	- 2	0.00	2769	182	-57	-0.31	1513
13	346	8	0.02	2489	366	- 9	-0.02	3175	125	6	0.05	1691
14	354	6	0.02	2794	357	- 6	-0.02	3506	131	-54.7	-0.42	1823
15	360	7	0.02	3196	251	6	0.02	3857	76.3	10	0.13	1933
16	367	7	0.02	3517	257	-77	-0.23	4117	86.3	-29.2	-0.34	2032
17	374	13	0.03	3930	180	33	0.18	4343	57.1	19.4	0.34	2092
18	387	8	0.02	4268	213	-46	-0.21	4563	76.5	-11.5	-0.15	2180
19	395	-14	-0.03	4703	167	-31	-0.18	4743	65.0	- 9.5	-0.14	2184
20	381	-42	-0.11	4780	136	-17	-0.12	4930	55.5	- 8.1	-0.14	2264
21	339	66	0.19	5455	119	1	0.00	5019	47.4	5.7	0.12	2296
22	405	-82	-0.20	5795	120	- 3	-0.02	5181	53.1	- 1.1	-0.02	2367
23	323	92	0.28	6216	117	-16	-0.14	5258	52.0	1.4	0.03	2399
24	415	-14	-0.25	6557	101	- 7.1	-0.07	5409	53.4	- 2.2	-0.04	2470
25	401	-17	-0.04	7011	93.9	- 5.1	-0.05	5463	51.2	- 7.3	0.14	2505
26	384			7370	88.8			5598	43.9			2571

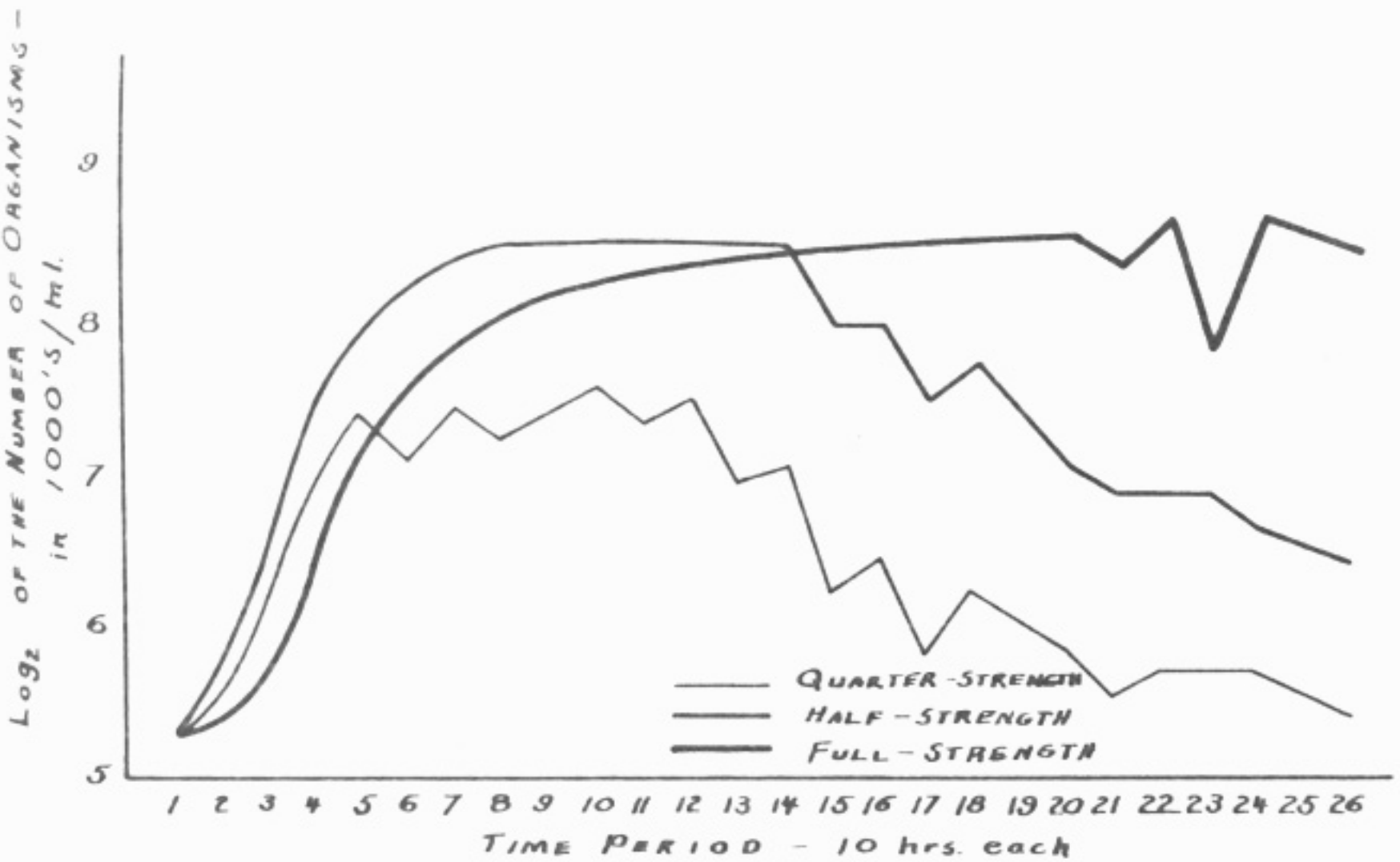

Figure 2. Growth of *Tetrahymena pyriformis* in different concentrations of medium of the same volume.

Beyond one-hundred-twenty hours of growth the oscillating population density of the quarter-strength medium continually decreases. The population density of the half-strength medium begins to oscillate and continually decrease near one-hundred-forty hours of growth. The full-strength medium begins oscillation of the population density near two-hundred hours of growth, and though not indicated by the graph, tends toward a more dense population during the experimental period of three-hundred-twenty hours.

At the same time periods after inoculation, the occupancy values of the half-strength medium exceed the occupancy values of the full-strength medium up to two-hundred-hours of growth. The occupancy values of the quarter-strength medium are greater than the occupancy values of the full-strength medium up to seventy hours of growth, but do not exceed the occupancy values of the half-strength medium during the entire experimental period.

The ratios of the maximum number of organisms per milliliter in the cultures of different volumes and the same concentration of medium indicate that the total number of organisms in the three different cultures was the same at the time the cultures reached their plateaus. The smaller culture approaches a maximum number of organisms per milliliter two times the maximum number of organisms per milliliter of the

culture twice as large. The larger culture reflects a maximum number of organisms per milliliter of the smallest culture. In the fifty milliliter cultures of different concentrations of medium, the maximal values obtained demonstrate that the full-strength medium and the half-strength medium reach plateaus similar to the plateaus first observed in the fifty milliliter cultures of the first experiment. The full-strength medium then continues upward to a maximum number of organisms equal to the maximum number of organisms attained in the fifty milliliter cultures in the first experiment, while the number of organisms in the half-strength medium rapidly declines.

The design of the experiment placed the total amount of energy available in each culture in proportion. In the twenty-five, fifty and seventy-five milliliter cultures the total energies available were respectfully one, two, and three times the total energy available in the twenty-five milliliter culture. In the full, half, and quarter-strength fifty milliliter cultures the total energies available were respectfully two, one, and one-half times the total energy available in the twenty-five milliliter culture; thus the cultures show three, two, one, and one-half times the total energy available in the twenty-five milliliter culture.

The seventy-five milliliter culture with three times the total energy available in the twenty-five milliliter culture develops a maximum population equal to only one-third of the maximum population of the twenty-five milliliter culture. In contrast the fifty milliliter culture with one-half of the total energy available in the twenty-five milliliter culture fails to develop to the maximum number of organisms expected in the fifty milliliter cultures, apparently due to the lack of sufficient total energy available. These observations indicate that a population size is independent of an energy excess.

One can visualize a population living in an optimal environment as rapidly expanding and filling any pre-imagined fixed space. Provided the environment will remain optimal, the fixed space will be the definite limiting factor of the population at the time the space becomes completely filled. The occupation of a population in a fixed space continually depletes the once optimal environment.

Theoretically at least, one can imagine that for a given set of cultural conditions a characteristic maximum area beneath the population growth curve will be expected. This characteristic area may be imagined as the total occupancy of the population.[1] Therefore as a population grows

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[1]Meglitsch, loc. cit.

and dies, the occupancy factor of the population will approach as a limit the characteristic area expected beneath the population growth curve. As the occupancy factor approaches this limit, one might well expect the course of the culture to be defined.

By plotting the specific growth rates of the population against the occupancy factors of the cultures for the same time periods; characteristic curves are obtainable as seen in figures 3 and 4. The maximum biotic potential on each graph is represented by the horizontal line parallel to the occupancy axis. The curves on the graph describe that part of the maximum biotic potential being realized, while the vertical distance between the horizontal line and the curve is a measure of the intensity of the environmental resistance.

The graphs indicate that during the very early periods of occupancy the environmental resistance is small and a high specific growth rate is obtained. As the occupancy of the cultures increases, the high specific growth rate is suppressed to a point near zero by a rapidly increasing environmental resistance. An approximate state of equilibrium between the biotic potential of the population and the environmental resistance is soon realized and represented in a gently sloping, almost horizontal line. At the time

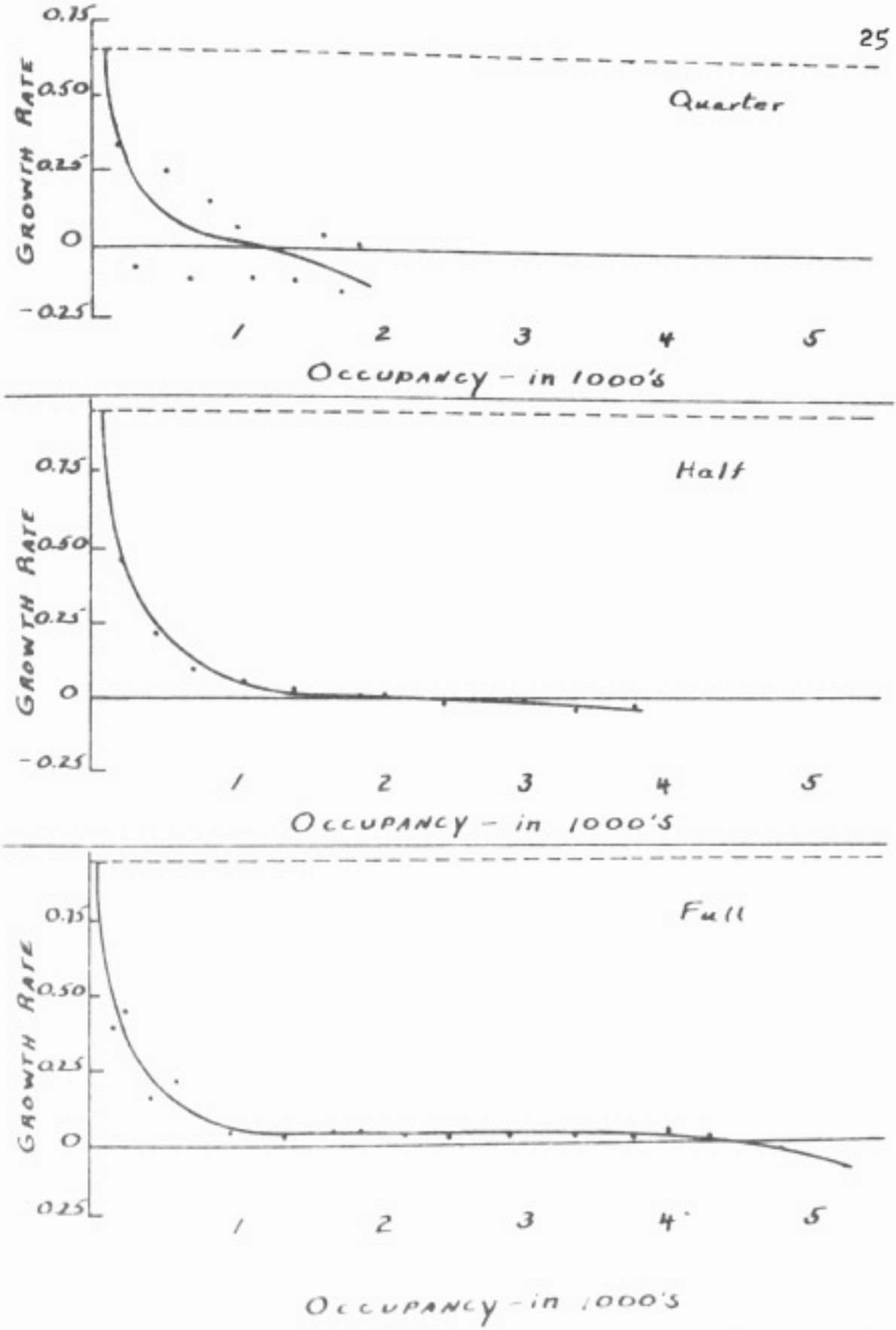

Figure 3. The effect of occupancy on the growth rate of *Tetrahymena pyriformis* in different concentrations of medium of equal volume.

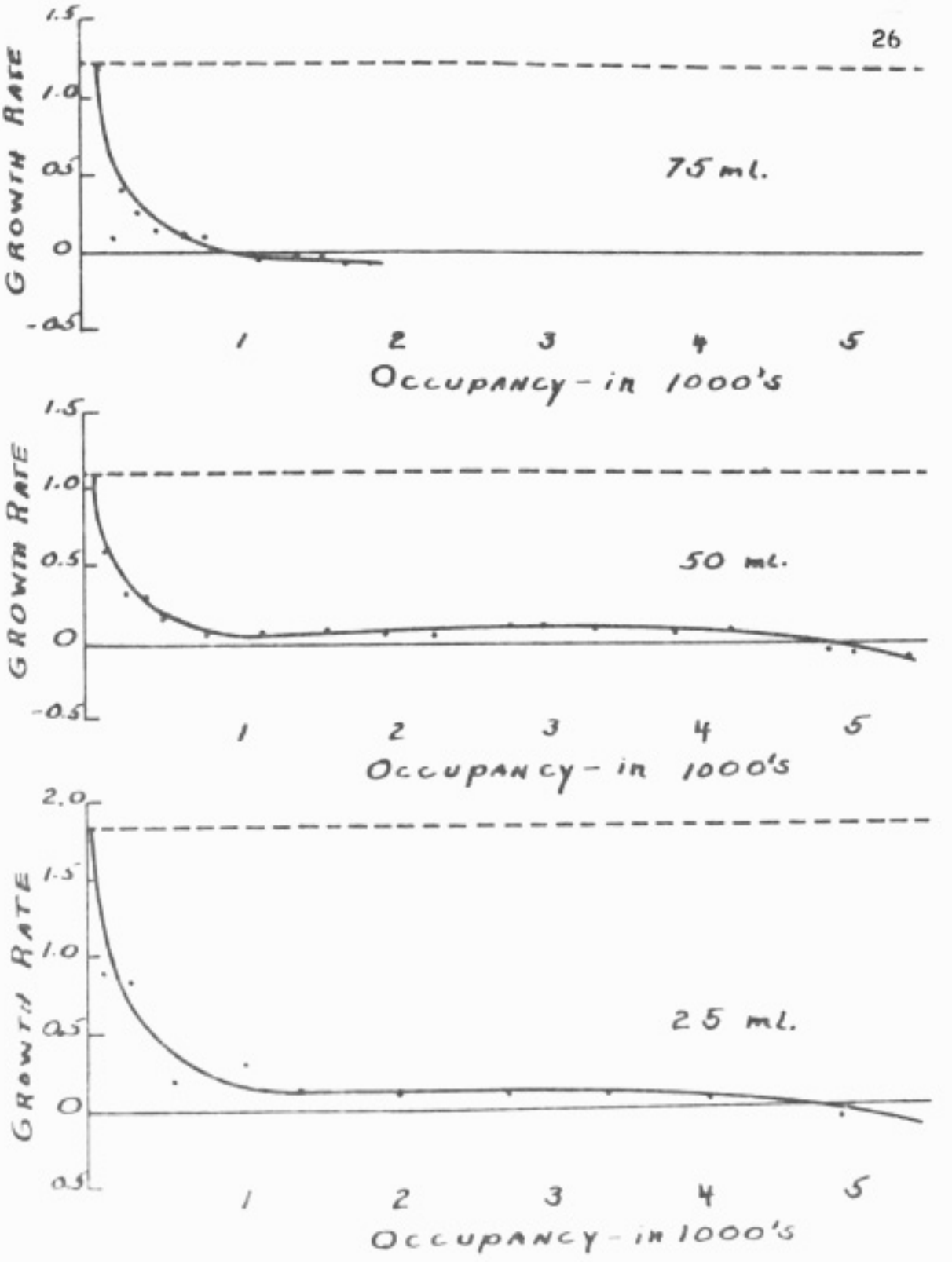

Figure 4. The effect of occupancy on the growth rate of *Tetrahymena pyriformis* in different volumes of medium of equal concentration.

the specific growth rate becomes zero, the biotic potential of the culture is equal to the environmental resistance.

The maximum specific growth rate conjointly with the rapidly increasing environmental resistance approximate the early effects of logarithmatic growth of the sigmoid growth curve. The plateau of the sigmoid growth curve is represented by the almost parallel, but gently sloping horizontal line. At the time the gently sloping horizontal line crosses the zero axis the upper aysmptote of the sigmoid growth is realized. The maximum growth rate observed establishes the beginning of sigmoid logarithmic growth; the gently sloping line marks the end of the maximum population growth at the time the line crosses the zero, or occupancy axis.

The environmental resistance measured as the difference of the observed maximum rate of increase and the observed actual rate of increase for a given period of time expresses accurately the reduction of the biotic potential due to all limiting factors. Occupancy, which embodies both the activities of synthesis and maintenance, is important in attempting to understand what aliquot of the total energy available is used in synthesis, and what aliquot is used in maintenance.

The probable relationship existing between the specific growth rate and occupancy is sufficient to signify that

further investigation should be conducted to establish the importance of the occupancy factor. A mathematical treatment of biological data is necessary to more clearly understand and accurately define the capacities of an organism to utilize the environment. Any progress in exploring the activities of synthesis and maintenance will be of value in constructing an integrated picture of the physiological condition of a population.

CHAPTER V

SUMMARY

By growing Tetrahymena pyriformis in an axenic medium, restricted growth curves are obtainable from the percent transmission of light through the culture medium, as recorded with the "Spectronic 20," Bausch and Lomb Colorimeter. This method permits an approximate determination of the specific growth rates and occupancy factors of a population, which can be used to interpret the biotic potential of a population and the environmental resistance of the culture medium as being dependent upon the occupancy factor.

The experiments demonstrate that the maximum population size is independent of an excess of the total energy available; but is dependent upon the intimate relationships existing between the population, the amount of available space, and a necessary minimum total energy available. The environmental resistance of the culture medium rapidly increases and establishes a state of near equilibrium with the biotic potential of the population, representing the plateau of the sigmoid growth curve. Maximum population size is achieved at the time the biotic potential of the population is suppressed to zero by the increasing environmental resistance extended by the

occupation of the population.

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